

WHAT IS CLAIMED IS:

1           1.     A method of testing the integrity of primers in a multiplex  
2     amplification reaction, the amplification reaction comprising primers sufficient to amplify at  
3     least two different target sequences, the method comprising,  
4                 providing in a mixture the primers and a single-stranded polynucleotide  
5     sequence comprising the sequences of the primers, subsequences of the primers at least five  
6     nucleotides long, or complements of the sequences of the primers;  
7                 amplifying the polynucleotide sequence; and  
8                 detecting the presence or absence of the amplified polynucleotide, thereby  
9     testing the integrity of the primers in the amplification reaction.

1           2.     The method of claim 1, wherein the target sequences are less than 50%  
2     identical to each other.

1           3.     The method of claim 1, wherein the single-stranded polynucleotide  
2     sequence is provided by denaturing a double-stranded polynucleotide.

1           4.     The method of claim 1, wherein the single-stranded polynucleotide  
2     sequence is a synthetic single-stranded polynucleotide.

1           5.     The method of claim 1, wherein the single-stranded polynucleotide  
2     sequence comprises the primer sequences.

1           6.     The method of claim 1, wherein the single-stranded polynucleotide  
2     sequence comprises subsequences of the primers at least five nucleotides long.

1           7.     The method of claim 1, wherein the single-stranded polynucleotide  
2     sequence comprises all subsequences of the primers that are nine nucleotides long.

1           8.     The method of claim 1, wherein the single-stranded polynucleotide  
2     comprises at least two subsequences of each primer, wherein the combination of the at least  
3     two subsequences contain every nucleotide of the primer sequence.

1           9.     The method of claim 1, wherein the single-stranded polynucleotide  
2     sequence comprises two subsequences of a primer sequence and at least the last two

3 nucleotides of a first subsequence are identical to the first at least two nucleotides of a second  
4 subsequence.

1 10. The method of claim 9, wherein at least the last five nucleotides of the  
2 first subsequence are identical to at least the first five nucleotides of the second subsequence.

1 11. The method of claim 1, wherein the mixture comprises at least a first,  
2 second, and third primer and the single-stranded polynucleotide sequence comprises the  
3 sequences of the at least first, second and third primer or subsequences at least five  
4 nucleotides long of the at least first, second and third primers.

1 12. The method of claim 1, wherein the mixture comprises primers  
2 sufficient to amplify at least three target sequences.

1 13. The method of claim 1, wherein the amplification of the target  
2 sequences is performed in the same reaction as the amplification of the single-stranded  
3 polynucleotide sequence.

1 14. The method of claim 1, wherein the mixture comprises a first primer  
2 pair and the single-stranded polynucleotide sequence comprises sequences, or complement  
3 thereof, of primers of the first primer pair oriented such that the first primer pair is capable of  
4 amplifying the remaining primer sequences, or subsequences thereof, in the single-stranded  
5 polynucleotide.

1 15. The method of claim 14, wherein the mixture comprises at least a  
2 second primer pair comprising a forward and a reverse primer, wherein the single-stranded  
3 polynucleotide sequence comprises sequences or subsequences of the at least second primer  
4 pair oriented such that the reverse primer sequence or subsequence is closer to the 5' end of  
5 the polynucleotide sequence than the forward primer sequence or subsequence.

1 16. The method of claim 15, wherein the single-stranded polynucleotide  
2 sequence comprises subsequences of the primers at least five nucleotides long.

1 17. The method of claim 15, wherein the single-stranded polynucleotide  
2 sequence comprises all subsequences of the primers that are nine nucleotides long.

1 18. A reagent kit, comprising

i. amplification reagents comprising primers sufficient to amplify at least two different target sequences;

ii. a polynucleotide sequence comprising the sequences of the primers or subsequences of the primers at least five nucleotides long; and

iii. at least one probe for detecting the polynucleotide sequence.

19. The reagent kit of claim 18, wherein the polynucleotide sequence is single-stranded.

20. The reagent kit of claim 18, wherein the polynucleotide sequence comprises the primer sequences.

21. The reagent kit of claim 18, wherein the polynucleotide sequence comprises subsequences of the primers at least five nucleotides long.

22. The reagent kit of claim 18, wherein the amplification reagents comprise a first primer pair and the single-stranded polynucleotide sequence comprises sequences, or complement thereof, of primers of the first primer pair oriented such that primer pair is capable of amplifying the remaining primer sequences, or subsequences thereof, in the single-stranded polynucleotide.

23. The reagent kit of claim 22, wherein the amplification reagents comprise at least a second primer pair comprising a forward and a reverse primer, wherein the single-stranded polynucleotide sequence comprises sequences or subsequences of the at least second primer pair oriented such that the reverse primer sequence or subsequence is closer to the 5' end of the polynucleotide sequence than the forward primer sequence or subsequence.

24. The reagent kit of claim 23, wherein the single-stranded polynucleotide sequence comprises subsequences of the primers at least five nucleotides long.

25. The reagent kit of claim 18, wherein the polynucleotide sequence comprises all subsequences of the primers that are nine nucleotides long.

26. The reagent kit of claim 18, wherein the polynucleotide sequence comprises two subsequences of a primer sequence and at least the last two nucleotides of a first subsequence are identical to the first at least two nucleotides of a second subsequence.

1                   27.     The reagent kit of claim 26, wherein at least the last five nucleotides of  
2     the first subsequence are identical to at least the first five nucleotides of the second  
3     subsequence.

1                   28.     The reagent kit of claim 18, wherein the kit comprises at least a first,  
2     second, and third primer and the single-stranded polynucleotide sequence comprises the  
3     sequences of the at least first, second and third primer or subsequences at least five  
4     nucleotides long of the at least first, second and third primers.

1                   29.     The reagent kit of claim 18, wherein the reagent kit comprises a first  
2     primer pair and the polynucleotide sequence comprises sequences of primers of the first  
3     primer pair oriented such that the first primer pair is capable of amplifying the remaining  
4     primer sequences, or subsequences thereof, in the single-stranded polynucleotide.

1                   30.     The reagent kit of claim 18, wherein the kit comprises at least a second  
2     primer pair comprising a forward and a reverse primer, and the single-stranded  
3     polynucleotide sequence comprises sequences or subsequences of the at least second primer  
4     pair oriented such that the reverse primer sequence or subsequence is closer to the 5' end of  
5     the polynucleotide sequence than the forward primer sequence or subsequence.

1                   31.     The reagent kit of claim 30, wherein the single-stranded polynucleotide  
2     sequence comprises subsequences of the primers at least five nucleotides long.

1                   32.     The reagent kit of claim 30, wherein the single-stranded polynucleotide  
2     sequence comprises all subsequences of the primers that are nine nucleotides long.